CLINICAL STUDY PROTOCOL D1015

A Multicenter, Open Label, Phase II Study of Bendamustine and Rituximab followed by 90-yttrium (Y) Ibritumomab Tiuxetan for Untreated Follicular Lymphoma (Fol-BRITe study)

Protocol Number: D1015

Indication: Untreated Follicular Lymphoma

Phase: II

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Protocol History:

Original	07 January 2010
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Amendment 7	26 May 2011
Amendment 8	29 February 2012
Amendment 9	06 March 2013
Amendment 10	19 October 2016
Amendment 11	06 July 2018

SCHEMA

Informed Consent and Pre-study Evaluations

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Eligibility Determination

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Registration

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Bendamustine + Rituximab (B-R) x 4 cycles

Rituximab	375mg/m^2	IV	Cycle 1 only: Day -7 (±1 day) Day 1 of every cycle	1 cycle = 28 days (±2 days) X 4 cycles
Bendamustine	90mg/m^2	IV	Days 1 and 2 of every cycle	

Restaging 4-6 weeks after last dose of B-R

(Although a maximum of 6 weeks is allowed, every effort should be made to restage as close to 4 weeks as possible.)

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Radioimmunotherapy with 90-yttrium (Y) Ibritumomab Tiuxetan (as per institutional procedures) 6-12 weeks after last dose of B-R

(Although a maximum of 12 weeks is allowed, every effort should be made to begin RIT as close to 6 weeks after the last cycle of B-R as possible.)

If participant meets criteria to receive RIT and platelet count is ≥ 150,000/mm^3:

Rituximab	250mg/m^2	IV	Day 1
Rituximab	250mg/ m^2	IV	Day 8 (±1 day)
Y-90 ibritumomab	0.4mCi/kg	IV	Within 4 hours of rituximab, give over 10 minutes

If participant meets criteria to receive RIT and platelet count is 100,000-149,000/mm^3:

Rituximab	250mg/m^2	IV	Day 1
Rituximab	250mg/ m^2	IV	Day 8 (±1 day)
Y-90 ibritumomab	0.3mCi/kg	IV	Within 4 hours of rituximab, give over 10 minutes

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(End of Treatment) Restaging 12-16 weeks after 90-yttrium (Y) Ibritumomab Tiuxetan

(Although a maximum of 16 weeks is allowed, every effort should be made to restage as close to 12 weeks as possible.)

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Long-term follow-up until relapse, progression, alternative therapy or death

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Effective 06July2018 – Annual follow-up until death

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1. STUDY OBJECTIVES

1.1. Primary Objective

1.1.1. To determine the complete response (CR) rate and overall response (OR) rate [CR + partial response (PR) rate] to a regimen of bendamustine and rituximab (B-R), followed by radioimmunotherapy (RIT) with 90-yttrium(Y) ibritumomab tiuxetan in subjects with untreated follicular lymphoma.

1.2. Secondary Objectives

1.2.1. To characterize the safety profile of bendamustine and rituximab followed by 90-yttrium(Y) ibritumomab tiuxetan in subjects with untreated follicular lymphoma
1.2.2. To determine the CR and OR rate after B-R
1.2.3. To determine the CR and OR rate after 90-yttrium(Y) ibritumomab tiuxetan specifically the conversions from PR to CR
1.2.4. To determine the progression-free survival (PFS)
1.2.5. To determine time to next treatment (TTNT)

1.3. Exploratory Objectives

1.3.1. To determine the molecular response after B-R as determined by qualitative polymerase chain reaction (PCR) of BCL2 from blood and bone marrow examination (required after B-R)
1.3.2. To determine the molecular response after 90-yttrium(Y) ibritumomab tiuxetan radioimmunotherapy from blood and bone marrow examination (required after RIT)

2. BACKGROUND

2.1 Follicular lymphoma

Non-Hodgkin's lymphomas (NHL) encompass a group of malignancies of lymphocytes that vary in their histologic appearance, aggressiveness and response to therapy.
According to the American Cancer Society, NHL is the 6th most common cancer, with more than 50,000 new cases per year. Follicular lymphoma (FL) is the 2nd most common type of NHL accounting for approximately 20% of newly diagnosed NHL. FL is considered an indolent, but incurable lymphoma. The goals of therapy are to treat symptomatic advanced stage disease to induce a maximum response with minimal toxicity. The optimal treatment of advanced stage follicular lymphoma (FL) remains to be determined. Combination chemotherapy is the standard frontline treatment option for this disease and the alkylating agent cyclophosphamide has been a common backbone in these combinations. The most common treatments for FL in the United States are rituximab combinations with chemotherapy such as cyclophosphamide, vincristine and prednisone (R-CVP) and cyclophosphamide, doxorubicin, vincristine and prednisone (R-CHOP). The NCCN guidelines also include fludarabine-based regimens, and radioimmunotherapy.

With the addition of immunotherapy (rituximab) to chemotherapy, the overall and complete response rates have improved.[1-6] Furthermore, there is suggestive evidence that overall survival may be improved.

Radioimmunotherapy (RIT) is also effective as salvage therapy for indolent lymphoma and transformed lymphoma.[7-9] In the first-line setting, RIT following chemotherapy can increase the CR rate and PFS.[10-12]

2.2 Bendamustine, Rituximab and 90-yttrium(Y) ibritumomab tiuxetan

2.2.1 Bendamustine and Rituximab

The chemical structure of bendamustine includes a bifunctional nitrogen mustard group, a benzimidazole ring, and a butyric acid side chain. It exhibits increased potency compared to other alkylators and has been shown to be active against cell lines which are resistant to other alkylating agents in pre-clinical experiments in vitro. These properties may be explained by a mechanism or mechanisms of action other than alkylation of DNA, possibly contributed by the presence of the benzimidazole ring and/or butyrate side chain or as a result of enhanced in vivo stability of the alkylating functionality.

Bendamustine has been used in Germany for over twenty years for the treatment of non-Hodgkin's lymphoma, chronic lymphocytic leukemia, Hodgkin's disease, multiple myeloma, and breast cancer. However, the drug has been relatively unknown in the United States until recent publications.[13]

Bendamustine as a single agent for patients who have rituximab-refractory non-Hodgkin's lymphoma has shown efficacy. Friedberg et al. showed an overall response rate of 77% (15% complete response, 19% unconfirmed complete response, and 43% partial response).[14] The median duration of response was 6.7 months, 9.0 months in patients with indolent disease, and 2.3 months in patients with transformed disease. The most frequent nonhematologic adverse events included nausea, vomiting, fatigue, constipation, anorexia, fever, cough, and diarrhea. Grade 3 or 4 reversible hematologic toxicities included neutropenia (54%), thrombocytopenia (25%), and anemia (12%). Overall, single-agent bendamustine produced durable objective responses with acceptable toxicity in heavily pretreated patients with rituximab-refractory indolent NHL.

Several randomized trials have shown that the addition of rituximab to chemotherapy results in higher response rates and longer time to progression and event-free survival in first-line FL or first relapse. For example, a randomized study of 8 cycles of cyclophosphamide, vincristine and prednisone (CVP) or R-CVP showed that the overall response rate (OR) and complete response rate (CR) were 81% and 41% in the R-CVP arm compared to 57% and 10% in the CVP only arm, respectively.[5, 6] Median time to treatment failure was 27 months in patients receiving R-CVP and 7 months in the CVP arm. This trial also showed a trend toward improvement in overall survival (OS) in the R-CVP arm. Similarly, the German Low-Grade Lymphoma Study Group (GLGLSG) showed in a randomized trial that R-CHOP was superior to CHOP with improved response rates (97% vs 90%), and longer time to treatment failure (not reached vs. 31 months).[4] Thus, it is widely accepted that rituximab added to chemotherapy is better than chemotherapy alone. The choice of a first-line regimen is highly variable, however. While fludarabine based-regimens are also highly effective in the first-line setting as well as in relapsing patients (Table 1), stem cell mobilization may be impaired for future autologous stem cell transplants.[1, 2, 15]

Due to clinical data showing activity of the combination of bendamustine plus rituximab in non-Hodgkin's lymphoma, a phase II study evaluated the combination of bendamustine plus rituximab in indolent non-Hodgkin's lymphoma including follicular and mantle cell histologies.[16] The study by Robinson et al. evaluated bendamustine plus rituximab in 67 adults with relapsed, indolent B-cell or mantle cell lymphoma without documented resistance to prior rituximab. Patients received rituximab 375 mg/ m^2 intravenously on day 1 and bendamustine 90 mg/m^2 intravenously on days 2 and 3 of each 28-day cycle for four to six cycles. The overall response rate was 92% (41% complete response, 14% unconfirmed complete response, and 38% partial response). Median duration of response was 21 months and median progression-free survival time was 23 months. Outcomes were similar for patients with indolent or mantle cell histologies. The primary toxicity was myelosuppression (grade 3 or 4 neutropenia, 36%; grade 3 or 4 thrombocytopenia, 9%).

For the first-line treatment of indolent lymphomas, the combination of bendamustine plus rituximab (B-R) was compared to R-CHOP. Rummel et al. showed that B-R is non-inferior to R-CHOP as first-line treatment of indolent lymphomas including follicular and mantle cell lymphomas, while showing a better tolerability profile such as less alopecia, and potentially less cardiotoxicity.[17] A total of 546 patients were randomized to B-R vs. CHOP-R x 6 cycles. The primary endpoint, progression-free survival (PFS), was not reached for the B-R arm and was 39 months in the R-CHOP arm. This difference was not statistically significant. The overall response rate was 94% vs 93% respectively, and the rate of CR was similar, 41% versus 33%. In this trial, 52% of patients had FL.

2.2.2 **90-yttrium(Y) ibritumomab tiuxetan**

Technical advances have made it possible to link radionuclides such as yttrium-90 (^{90}Y) to monoclonal antibodies to specifically target radiation to lymphoma cells. Yttrium-90 is a beta-emitting radionuclide that delivers 90% of its radiation (2.3 MeV) over a mean path length of 5 mm and has a half-life of 64 hours. These characteristics are particularly advantageous for treating bulky, poorly vascularized tumors and those with heterogeneous antigen expression.

Ibritumomab tiuxetan (Zevalin; Spectrum Pharmaceuticals) is a short-course radioimmunotherapy that uses immunobiologic and radiolytic mechanisms of action to destroy both dividing and nondividing tumor cells. Ibritumomab is the murine, parent anti-CD20 antibody that was engineered to develop rituximab. Tiuxetan is a linker/chelator covalently attached to ibritumomab. Ibritumomab tiuxetan can chelate indium-111 (^{111}In) for imaging or ^{90}Y for therapy. Thus, the antibody specifically targets radiation to $CD20^+$ cells while sparing normal nonlymphoid cells.

Because rituximab immunotherapy is widely used to treat patients with NHL, a critical issue is whether patients treated previously with rituximab can respond to ibritumomab tiuxetan. This study tested the hypothesis that tumor resistance to an unconjugated anti-CD20 antibody alone can be overcome by subsequent CD20-directed radioimmunotherapy.

Witzig et al. showed that ibritumomab tiuxetan radioimmunotherapy is effective in rituximab-refractory patients.[9] Eligible patients were refractory to rituximab; this was defined as no objective response to rituximab (375 mg/m^2 weekly for 4 weeks) or time to progression (TTP) of = ≤6 months. The ibritumomab tiuxetan treatment regimen consisted of pretreatment with rituximab (250 mg/m^2) intravenously on days 1 and 8 to deplete peripheral blood B cells, then yttrium-90 ibritumomab tiuxetan (0.4 mCi/kg; maximum, 32 mCi) intravenously on day 8, administered on an outpatient basis. An imaging/dosimetry dose of indium-111 ibritumomab tiuxetan (5 mCi) was injected after rituximab (day 1) in 28 patients. (The indium-111 and dosimetry bioscan are no longer required as per the FDA and revised package insert 11/2011). Fifty-seven patients were treated. The median age was 54 years, 74% had tumors ≥ 5 cm, and all were extensively pretreated (median, four prior therapies; range, one to nine). The estimated radiation-absorbed doses to healthy organs were below the study-defined limit in all patients studied with dosimetry. The overall response rate for the 54 patients with follicular NHL was 74% (15% complete responses and 59% partial responses). The Kaplan-Meier-estimated TTP was 6.8 months (range, 1.1 to ≥ 25.9 months) for all patients and 8.7 months for responders. Adverse events were primarily hematologic; the incidence of grade 4 neutropenia, thrombocytopenia, and anemia was 35%, 9%, and 4%, respectively. The only significant toxicity was hematologic.

2.3 Rationale of combining bendamustine and rituximab with consolidation 90-yttrium(Y) ibritumomab tiuxetan

As mentioned above, the combination of bendamustine plus rituximab (B-R) appears to be non-inferior to R-CHOP as first-line treatment of indolent lymphomas including follicular and mantle cell lymphomas, while showing a better tolerability profile such as less alopecia, and potentially less cardiotoxicity, making it a rational choice for first line treatment of FL.[17]

When given after chemotherapy, radioimmunotherapy can convert partial responses to complete responses and can prolong the PFS. The Follicular Lymphoma Ibritumomab tiuxetan (FIT) trial of consolidation Yttrium-90-Ibritumomab tiuxetan versus no additional therapy after first remission in advanced follicular lymphoma showed a prolongation of PFS (36 versus 13 months) in the RIT arm.[12] The PFS was prolonged regardless of PR or CR after first-line therapy. The primary treatment included CVP, CHOP, fludarabine-based, and chlorambucil, with the minority of patients receiving rituximab. The results also showed that RIT converted 77% patients from PR to CR/unconfirmed CR (CRu).

An abbreviated course of CHOP-R followed by RIT has shown promise in patients with follicular lymphoma in a phase II trial reported recently.[11] Of the 60 patients entering this trial, 55 patients completed all protocol therapy. The median follow up was 19.7 months (range, 0.26-35.9 months). For intent-to-treat analysis, the complete response (CR) rate after CHOP-R, as assessed by CT and PET imaging, was 40% and 46%, respectively. After RIT, the CR rate improved, as assessed by CT and PET imaging, to 82% and 89%, respectively.

In this current study, we propose a first-line regimen for untreated FL using bendamustine and rituximab (B-R) (bendamustine 90 mg/m^2 on days 1 and 2 and Rituximab 375mg/m^2 on Day 1 of a 28-day [±2 days] cycle) x 4 cycles followed by RIT; Zevalin (formerly Biogen

Idec/Cell Therapeutics, now Spectrum).

The advantage of this treatment is that B-R has a better side effect profile including significantly less alopecia and less infectious complications. Currently bendamustine is not FDA-approved for first-line therapy for follicular lymphoma. 90-yttrium(Y) ibritumomab tiuxetan (Zevalin) radioimmunotherapy is FDA approved for patients with previously untreated follicular non-Hodgkin's Lymphoma (NHL), who achieve a partial or complete response to first-line chemotherapy. Evidence suggests that consolidation with RIT leads to a longer PFS. Since this specific combination has not been utilized in the first-line treatment of FL, it warrants investigation in the current study.

This trial will begin to establish a standard of care for the first-line treatment of follicular lymphoma. We hypothesize that bendamustine plus rituximab followed by RIT will contribute to among the highest CR rates seen in follicular lymphoma with relatively low toxicity. Based on the results of this trial, we would aim to open a larger trial for follicular lymphoma in a cooperative group setting, i.e. CALGB.

2.4 **Correlative Studies Background**

The BCL2 gene-Jh rearrangement is the common abnormality in FL t(14;18). This can be assessed by various PCR techniques.[18, 19]

Patients can be assessed for this molecular abnormality in their bone marrow at baseline and following therapy. For instance in a similar Southwest Oncology Group study of chemotherapy followed by radioimmunotherapy using tositumomab/iodine I-131 tositumomab (Bexxar) for follicular lymphoma, patients were asked to undergo serial bone marrow aspirations at study entry, 4 weeks after the sixth cycle of CHOP (just before tositumomab/iodine I-131 tositumomab), and after tositumomab/iodine I-131 tositumomab for PCR testing.[20] The mononuclear cell fraction was isolated from marrow aspirates by Ficoll-Hypaque sedimentation and cryopreserved for subsequent batch analysis using a double nested PCR assay to detect the major breakpoint region and the minor cluster region of the *BCL2* gene using the method of Gribben.[18, 19] Samples were initially analyzed by fragment size using ethidium bromide gel electrophoresis of the PCR product and then transferred to nitrocellulose membranes for confirmation of the identity of the *BCL2* translocation by Southern blotting. The adequacy of samples was demonstrated using beta-globin as a positive control housekeeping gene. Patients were considered to have attained a molecular remission if their marrow sample at study entry contained a detectable t(14;18) translocation that became undetectable after protocol treatment.

3. ELIGIBILITY CRITERIA

3.1 Inclusion Criteria

3.1.1 Previously untreated, histologically confirmed follicular lymphoma classification grade 1, 2 or 3a (>15 centroblasts per high power field with centrocytes present)

3.1.2 Ann Arbor stages of II to IV with either symptomatic or bulky disease (>5 cm); or disease progression

3.1.3 Male or female subject 18 years of age or older

3.1.4 ECOG performance status $\leq$2

3.1.5 Patients must have normal organ and marrow function as defined below:
Adequate hematologic function
- Absolute neutrophil count (ANC) $\geq$1,000/mm^3
- Platelet count $\geq$100,000/mm^3
- Patients with an ANC less than 1,000/mm^3 and/or platelets below 100,000/mm^3 are still eligible for study entry as long as there is >50% bone marrow involvement with lymphoma

Adequate hepatic function
- Total bilirubin within normal institutional limits
- AST(SGOT) $\leq$2.5 X institutional upper limit of normal
- ALT(SGPT) $\leq$2.5 X institutional upper limit of normal

Adequate renal function
- Calculated creatinine clearance >40 mL/min/1.73m^2
  Creatinine clearance should be calculated using the Cockcroft-Gault Equation (Appendix F) or per institutional standard

3.1.6 Measureable disease with at least one lesion measuring $\geq$ 2 cm in its greatest transverse diameter

3.1.7 Female subjects of childbearing potential must have a negative pregnancy test (urine or serum b-HCG) at screening. Both male and female subjects must employ effective contraceptive measures prior to the start of therapy until 12 months after the last dose of study drug. Men must agree not to father a child and agree to use effective birth control during therapy and for 12 months after the last dose of study drug, even if they have had a successful vasectomy, if their partner is of childbearing potential.

3.1.8 Voluntary written informed consent must be given before performance of any study-related procedure, with the understanding that consent may be withdrawn by the subject at any time without prejudice to future medical care. The subject must have the ability to understand and the willingness to sign a written informed consent document.

3.3 Exclusion Criteria

3.3.1 Patients who have had prior chemotherapy or immunotherapy

3.3.2 Patients receiving any other investigational agents

3.3.3 Prior chemotherapy or monoclonal antibody therapy; prior radiation will be allowed if <25% of active bone marrow was exposed

3.3.4 Patients with primary CNS lymphoma

3.3.5 Patients with known HIV

3.3.6 Treatment with therapeutic doses of systemic steroids within 4 weeks of beginning study treatment (cycle 1, day -7); topical use of corticosteroids and systemic replacement of corticosteroids for adrenal insufficiency are allowed

3.3.7 Patients who have had malignant pleural, pericardial or peritoneal effusions at any time

3.3.8 Patients with a known history of myelodysplastic syndrome (MDS) or found to have MDS on review of the staging bone marrow aspirate and biopsy

3.3.9 Uncontrolled intercurrent illness including, but not limited to, ongoing or active infection, symptomatic congestive heart failure, unstable angina pectoris, cardiac arrhythmia, or psychiatric illness/social situations that would, in the judgment of the investigator, limit compliance with study requirements

3.3.10 Pregnant or lactating female subjects

3.3.11 Concurrent active malignancy other than lymphoma or history of invasive malignancy within the past 5 years. The only exception is completely excised, non-melanoma skin cancer

3.3.12 Known Hepatitis B and/or Hepatitis C Infection

3.3.13 Any other condition, that in the judgment of the investigator places the patient at unacceptable risk if he/she were to participant in the study

3.4 Inclusion of Women and Minorities

Both men and women and members of all races and ethnic groups are eligible for this trial.

4. REGISTRATION PROCEDURES

4.1 General Guidelines

Each participant must sign and date an informed consent form before undergoing any study-specific procedures. Contact the Project Manager at the Norris Cotton Cancer Center Clinical Research Office once consent is signed. The Project Manager will assign a participant identification number. Once a participant has completed all pre-treatment evaluations and eligibility criteria have been met, participants will be registered to the study.

The registration form, PI or designee signed eligibility checklist, primary source documentation including pathologic documentation of the lymphoma, bone marrow examination, and staging studies should be faxed or emailed to the Project Manager. The Project Manager will send a confirmatory email that these materials have been received.

Eligibility criteria will be verified by the Dartmouth PI or designee at the time of registration. Once the participant is determined by the PI or designee to be eligible, the subject will be registered. A completed registration form will be sent confirming registration. Each participant will then be entered into Velos by the Dartmouth Project Manager.

4.2 Registration Process

To register a patient, the following documents need to be completed and faxed (603-650-7799) or e-mailed to the Project Manager:

- Investigator signed eligibility checklist
- Lymphoma pathology report, bone marrow examination (when available), and staging studies
- Copy of required laboratory reports, including creatinine clearance calculation
- Patient Enrollment Form from Spectrum Pharmaceuticals
- Completed registration form

Within 24 hours the Project Manager will:

- Assign a participant study ID number
- Fax or e-mail the participant study ID number to the participating site
- Verify eligibility with the Dartmouth PI or designee
- Register the participant to the study
- Contact the participating site and confirm registration

5. TREATMENT PLAN

5.0 Study design

This multicenter, open label, single arm clinical trial has been designed to investigate bendamustine plus rituximab followed by 90-yttrium(Y) ibritumomab tiuxetan in patients with untreated follicular lymphoma requiring treatment. Upon successful completion of all screening procedures and registration, participants will be treated following the schema outlined below:

In cycle 1 only, participants will receive rituximab 375mg/m^2 on day -7 ($\pm$1 day). For every cycle, participants will receive bendamustine 90 mg/m^2 and rituximab 375 mg/m^2 on day 1 and bendamustine 90 mg/m^2 on day 2. A cycle will be 28 days ($\pm$2 days) and participants will be treated for a total of 4 cycles.

Participants, who achieve at least a partial response (PR), achieve a platelet count $\geq$100,000/mm^3, an ANC $\geq$ 1500/mm^3, and bone marrow infiltration less than 25% at restaging will be eligible for consolidation radioimmunotherapy (RIT) with 90-yttrium(Y) ibritumomab tiuxetan. RIT treatment will begin 6-12 weeks after the last cycle of B-R. Although a maximum of 12 weeks is allowed, every effort should be made to begin RIT as close to 6 weeks after the last cycle of B-R as possible. Participants who either have stable disease (SD) or progression of disease or do not complete 4 cycles of bendamustine and rituximab treatment will not be eligible to receive RIT.

The ibritumomab tiuxetan therapeutic regimen is given in 2 steps: Step 1 includes one infusion of rituximab on day 1. (Step 1 no longer requires the use of indium-111 or the dosimetric bioscan as of 11/2011 as per the FDA and new package insert 11/2011.) (http://zevalin.com/v3/pdf/Zevalin_Package_Insert.pdf) Step 2 is given on day 8 ($\pm$1 day) and consists of a second infusion of rituximab preceding Y-90 ibritumomab tiuxetan. Rituximab is administered to deplete peripheral blood CD20+ B-cells and to optimize biodistribution.

Following RIT, a weekly CBC w/diff including platelet count is required until count recovery. Every 4 weeks (starting from day 1) a physical exam, CBC w/ diff, serum chemistry, vitals, ECOG, and adverse event evaluation is required. Count recovery following RIT is defined as ANC $\geq$1000/mm^3 and platelet count $\geq$100,000/mm^3.

5.1 Agent Administration

Treatment will be administered on an outpatient basis. Reported adverse events and potential risks for bendamustine, rituximab and 90-yttrium(Y) ibritumomab tiuxetan are described in Section 7. Appropriate dose modifications of bendamustine, rituximab and 90-yttrium(Y) ibritumomab tiuxetan are described in Section 6. No investigational or commercial agents or therapies other than those described below may be administered with the intent to treat the patient's malignancy.

<table>
<tr><th colspan="6">REGIMEN DESCRIPTION</th></tr>
<tr><th colspan="6">Bendamustine and Rituximab</th></tr>
<tr><th>Agent</th><th>Premedications; Precautions</th><th>Dose</th><th>Route, Rate</th><th>Schedule</th><th>Cycle Length</th></tr>
<tr><td>Rituximab</td><td>Acetaminophen 650mg PO, diphenhydramine 25-50 mg PO or IV, dexamethasone 8-12mg PO or IV</td><td>375mg/m^2</td><td>IV , Rate: Administer per institutional guidelines</td><td>Cycle 1 only: Day -7 (±1 day)
Day 1 of every cycle</td><td rowspan="2">1 cycle = 28 days (±2 days)</td></tr>
<tr><td>Bendamustine</td><td>Serotonin-receptor antagonist (ondansetron, granisetron, or equivalent antiemetic) is allowed</td><td>90mg/m^2</td><td>IV, Rate: Administer per institutional guidelines</td><td>Days 1 and 2 of every cycle</td></tr>
<tr><th colspan="6">Radioimmunotherapy with Y-90 ibritumomab tiuxetan: 6-12 weeks after last dose of B-R</th></tr>
<tr><td>Rituximab and Y-90 ibritumomab tiuxetan

(refer to package insert for Y-90 ibritumomab tiuxetan for details regarding administration, or refer to institutional protocols.)</td><td colspan="5">Step 1: Day 1: Rituximab 250 mg/m^2 IV is administered first (see Rituximab package insert).

Step 2: Day 8 (±1 day): a second dose of Rituximab 250 mg/m^2 IV is given.

If platelet count ≥ 150,000/mm^3: Within 4 hours of completing the rituximab infusion, give Y-90 ibritumomab tiuxetan 0.4 mCi/kg (14.8 MBq/kg) IV over 10 minutes. The dose administered should be within 10% of the actual prescribed dose of Y-90 ibritumomab tiuxetan.
If platelet count 100,000—149,000/mm^3: Within 4 hours of completing the rituximab infusion, give Y-90 ibritumomab tiuxetan 0.3 mCi/kg (11.1 MBq/kg) IV over 10 minutes. The dose administered should be within 10% of the actual prescribed dose of Y-90 ibritumomab tiuxetan.

The total Y-90 dose should not exceed 32 mCi (1184 MBq) regardless of the patient’s body weight.</td></tr>
</table>

For rituximab dosing and administration, rituximab will be rounded to the nearest 10 mg.

5.2 General Concomitant Medication and Supportive Care Guidelines

No formal clinical assessments of pharmacokinetic drug-drug interactions between bendamustine and other drugs have been conducted. Inhibitors of CYP1A2 (e.g., fluvoxamine, ciprofloxacin) have potential to increase plasma concentrations of bendamustine and decrease plasma concentrations of active metabolites. Inducers of CYP1A2 (e.g., omeprazole, smoking) have potential to decrease plasma concentrations of bendamustine and increase plasma concentrations of its active metabolites. Caution should be used if concomitant treatment with CYP1A2 inhibitors or inducers is needed.

Prophylactic use of G-CSF or GM-CSF is discouraged. They are allowed in participants who

demonstrate the need for bone marrow support based on occurrence of prolonged neutropenia (Grade 4 leukopenia lasting at least one week, failure of WBC count to recover to at least a grade 1 toxicity by the next scheduled dose, or the occurrence of febrile neutropenia in a prior cycle of treatment.)

Use of G-CSF or GM-CSF should not be used as a substitute for dose reduction.

5.3 **Duration of Therapy**

In the absence of disease progression or unacceptable toxicity as deemed by the treating physician, treatment will continue for the planned duration of protocol therapy. Any participant with documented disease progression will be taken off of study treatments and removed from the protocol.

Other conditions for withdrawal:
If at any time the constraints of this protocol are detrimental to the participant's health and/or the participant no longer wishes to continue protocol therapy, protocol therapy shall be discontinued. In this event, the Dartmouth PI shall be notified, and the reason(s) for discontinuation of therapy should be documented in the Case Report Form.

5.4 **Duration of Follow Up**

Participants who receive any amount of study treatment will be followed every 3 months (+/- 2 weeks) with a physical exam, vital signs, weight, ECOG, CBC with diff, platelets, and serum chemistry for the first 2 years. CT scans will be performed every 6 months for the first 2 years. Years 3 through 10, participants will be followed annually. If the participant receives alternative treatment, data will be collected on the type of alternative treatment and date received. This will allow determination of time to next treatment. Follow up will occur for a maximum of 10 years from study entry or death – whichever occurs first. For those who do not receive Y90 ibritumumab tiuxetan but then receive maintenance rituximab, this does not constitute a new regimen, and patients should be followed for a maximum of 10 years from study entry or death – whichever occurs first. Rituximab should be recorded in the concomitant medications.

6. DOSING DELAYS/DOSE MODIFICATIONS

6.1 Starting Dose and Dose Modifications for Bendamustine

The starting dose of bendamustine will be 90 mg/m^2 IV infusion over 30-60 minutes. The dose should be calculated based on the BSA of the patient on day 1 of every cycle.

Participants who experience Grade 4 hematologic or Grade 3-4 non-hematologic toxicity after a cycle of B-R therapy will have their dose of bendamustine decreased to 60 mg/m^2 IV for the remaining cycles.

Participants who continue to experience Grade 4 hematologic or Grade 3-4 non-hematologic toxicity at this reduced dosage level should discontinue B-R. Any participant that does not meet criteria to receive RIT after 12 weeks from the last dose of B-R, will removed from study treatment and will be considered off study. (see section 5.4).

Dose Level	Bendamustine Dose
Baseline	90 mg/m^2
-1	60 mg/m^2

Prophylactic use of G-CSF or GM-CSF is discouraged. They are allowed in participants who demonstrate the need for bone marrow support based on occurrence of prolonged neutropenia (Grade 4 leukopenia lasting at least one week, failure of WBC count to recover to at least Grade 1 toxicity by the next scheduled dose, or the occurrence of febrile neutropenia in a prior cycle of treatment.)

Use of G-CSF or GM-CSF should not be seen as a substitute for dose reduction.

6.2 Inclusion Criteria Prior to RIT

6.2.1 Patients must have achieved at least a partial response (PR), an ANC >1500/mm^3, a platelet count >100,000/mm^3 and less than 25% bone marrow involvement with lymphoma prior to the start of 90-yttrium (Y) ibritumomab tiuxetan.

6.2.2 Female subjects of childbearing potential must have a negative pregnancy test (urine or serum b-HCG) within 1 week prior to the start of treatment with Y-90 ibritumomab Tiuxetan.

6.2.3 Patients must complete 4 cycles of bendamustine and rituximab treatment per protocol.

6.3 Starting Dose of 90-yttrium(Y) ibritumomab tiuxetan

90-yttrium(Y) ibritumomab tiuxetan shall be given 6-12 weeks after completion of B-R provided the participant has achieved a platelet count of ≥100,000/mm^3, an ANC ≥ 1500/mm^3, and bone marrow infiltration less than 25%. Although a maximum of 12 weeks is allowed, every effort should be made to begin RIT as close to 6 weeks after the last cycle of B-R as possible.

On the actual day of RIT, for platelet counts ≥ 150,000/mm^3, Y-90 ibritumomab tiuxetan dose will be 0.4 mCi/kg (14.8 MBq/kg).

On the actual day of RIT, for platelets count 100,000-149,000/mm^3, Y-90 ibritumomab tiuxetan dose will be 0.3 mCi/kg (11.1 MBq/kg).

6.4 **Postponement of Bendamustine and Rituximab Treatment**

The prescribed time interval between each cycle of rituximab plus bendamustine is 28 days (±2 days). Non-hematologic toxicities must have recovered to ≤ grade 1 or to baseline before receiving the next treatment course. At the start of cycles 2 through 4, the absolute neutrophil count must be ≥1,000/mm^3 and the platelet count must be ≥ 75,000/mm^3 to proceed with treatment (unless the subject has documented ≥50% marrow involvement with lymphoma). If recovery criteria are not met within 2 weeks following the prescribed start of a treatment cycle (i.e. after 2 week delay), the patient will be re-evaluated by the investigator, and a decision will be made regarding further treatment with B-R.

6.5 **Ancillary Therapy**

No other anti-tumor treatment is allowed during the course of the study. The investigator may prescribe supportive treatment for adverse events following the evaluation of the causal relationship of the symptom(s) to the study drug. Onset and duration of supportive treatment, including pre-infusion medications, should be recorded on the concomitant medication log and in the CRF.

7. ADVERSE EVENTS: LIST AND REPORTING REQUIREMENTS

Adverse event (AE) recording and reporting is a routine part of every clinical trial. All AEs and SAEs, whether reported by the participant or noted by authorized study personnel, will be recorded in the participant's medical record, on the AE or SAE log, and the case report forms. AE recording will begin on the first day of treatment (Cycle 1 Day -7) and end at the EOT visit. An ongoing AE will be followed until it returns to baseline. Abnormal lab values, even if they are expected, must be recorded appropriately as adverse events. Each site must record the onset and duration of each hematologic AE.
The following list of AEs (Section 7.1) and the characteristics of an observed AE (Section 7.2) may assist with determination of SAE reporting.

7.1 **Adverse Events**

7.1.1 Clinical Trials Experience in NHL with Bendamustine

The most common nonhematologic adverse reactions (≥30%) were nausea (75%), fatigue (57%), vomiting (40%), diarrhea (37%) and pyrexia (34%). The most common non-hematologic Grade 3 or 4 adverse reactions (≥5%) were fatigue (11%), febrile neutropenia (6%), and pneumonia, hypokalemia and dehydration, each reported in 5% of patients. (Please refer to the package insert of Treanda®).

In addition the following adverse events have been reported.

Tumor Lysis Syndrome
Tumor lysis syndrome associated with bendamustine treatment has been reported in patients in clinical trials and in spontaneous reports. The onset tends to be within the first treatment cycle of bendamustine and, without intervention, may lead to acute renal failure and death. Preventive measures include maintaining adequate volume status, and close monitoring of serum chemistry, particularly potassium and uric acid levels. Allopurinol has also been used prior to or at the beginning of bendamustine therapy. However, there may be an increased risk of severe skin toxicity when bendamustine and allopurinol are administered concomitantly.

Skin Reactions
Skin reactions have been reported in clinical trials and postmarketing spontaneous reports. These events have included rash, toxic skin reactions, and bullous exanthema. Some events occurred when bendamustine was given in combination with other anticancer agents, so the precise relationship of the skin reactions to bendamustine treatment is uncertain. In a study of bendamustine (90 mg/m^2) in combination with rituximab (study SDX-105-02), 1 case of toxic epidermal necrolysis (TEN) occurred. TEN has been associated with treatment with rituximab. Spontaneous reports of SJS and TEN, some fatal, have been reported when bendamustine was administered concomitantly with allopurinol and other medications known to cause these syndromes. The relationship to bendamustine cannot be determined.

When skin reactions occur, they may be progressive and increase in severity with further treatment. Therefore, patients with skin reactions should be monitored closely. If skin reactions are severe or progressive, bendamustine treatment should be withheld or discontinued.

Comprehensive Non-Hematologic Toxicities Occurring in at least 5% of NHL patients treated with bendamustine.

Table 3: Non-Hematologic Adverse Reactions Occurring in at Least 5% of NHL Patients Treated with TREANDA by System Organ Class and Preferred Term (N=176)

System organ class Preferred term	Number (%) of patients*	
	All Grades	Grade 3/4
Total number of patients with at least 1 adverse reaction	176 (100)	94 (53)
Cardiac disorders		
Tachycardia	13 (7)	0
Gastrointestinal disorders		
Nausea	132 (75)	7 (4)
Vomiting	71 (40)	5 (3)
Diarrhea	65 (37)	6 (3)
Constipation	51 (29)	1 (<1)
Stomatitis	27 (15)	1 (<1)
Abdominal pain	22 (13)	2 (1)
Dyspepsia	20 (11)	0
Gastroesophageal reflux disease	18 (10)	0
Dry mouth	15 (9)	1 (<1)
Abdominal pain upper	8 (5)	0
Abdominal distension	8 (5)	0
General disorders and administration site conditions		
Fatigue	101 (57)	19 (11)
Pyrexia	59 (34)	3 (2)
Chills	24 (14)	0
Edema peripheral	23 (13)	1 (<1)
Asthenia	19 (11)	4 (2)
Chest pain	11 (6)	1 (<1)
Infusion site pain	11 (6)	0
Pain	10 (6)	0
Catheter site pain	8 (5)	0
Infections and infestations		
Herpes zoster	18 (10)	5 (3)
Upper respiratory tract infection	18 (10)	0
Urinary tract infection	17 (10)	4 (2)
Sinusitis	15 (9)	0
Pneumonia	14 (8)	9 (5)
Febrile Neutropenia	11 (6)	11 (6)
Oral Candidiasis	11 (6)	2 (1)
Nasopharyngitis	11 (6)	0
Investigations		
Weight decreased	31 (18)	3 (2)
Metabolism and nutrition disorders		
Anorexia	40 (23)	3 (2)
Dehydration	24 (14)	8 (5)
Decreased appetite	22 (13)	1 (<1)
Hypokalemia	15 (9)	9 (5)
Musculoskeletal and connective tissue disorders		
Back pain	25 (14)	5 (3)
Arthralgia	11 (6)	0
Pain in extremity	8 (5)	2 (1)
Bone pain	8 (5)	0
Nervous system disorders		
Headache	36 (21)	0
Dizziness	25 (14)	0
Dysgeusia	13 (7)	0
Psychiatric disorders		
Insomnia	23 (13)	0
Anxiety	14 (8)	1 (<1)
Depression	10 (6)	0
Respiratory, thoracic and mediastinal disorders		
Cough	38 (22)	1 (<1)
Dyspnea	28 (16)	3 (2)
Pharyngolaryngeal pain	14 (8)	1 (<1)
Wheezing	8 (5)	0
Nasal congestion	8 (5)	0
Skin and subcutaneous tissue disorders		
Rash	28 (16)	1 (<1)
Pruritus	11 (6)	0
Dry skin	9 (5)	0
Night sweats	9 (5)	0
Hyperhidrosis	8 (5)	0
Vascular disorders		
Hypotension	10 (6)	2 (1)

*Patients may have reported more than 1 adverse reaction.

NOTE: Patients counted only once in each preferred term category and once in each system organ class category.

7.1.2 Adverse Event List for 90-yttrium(Y) ibritumomab tiuxetan

The following adverse reactions are possible with 90-yttrium(Y) ibritumomab tiuxetan. (Please refer to the package insert of Zevalin®).

- Serious Infusion Reactions
- Prolonged and Severe Cytopenias
- Severe Cutaneous and Mucocutaneous Reactions
- Secondary Leukemia and Myelodysplastic Syndrome

The most common adverse reactions of the 90-yttrium(Y) ibritumomab tiuxetan therapeutic regimen are: neutropenia, thrombocytopenia, anemia, gastrointestinal symptoms (nausea, vomiting, abdominal pain, and diarrhea), increased cough, dyspnea, dizziness, arthralgia, anorexia, anxiety, and ecchymosis.

The most serious adverse reactions of the 90-yttrium(Y) ibritumomab tiuxetan therapeutic regimen are prolonged and severe cytopenias, infections (predominantly bacterial in origin), hemorrhage while thrombocytopenic, severe cutaneous and mucocutaneous reactions, infusion reactions (bronchospasm and angioedema), myeloid malignancies and myelodysplasias.

Comprehensive Non-Hematologic Toxicities Occurring in at least 5% of NHL patients treated with 90-yttrium(Y) ibritumomab tiuxetan.

Incidence of Adverse Reactions in ≥ 5 % of Patients Receiving the Zevalin therapeutic regimen[†] (N = 349)

	All Grades %	Grade 3/4 %
Any Adverse Reaction	**99**	**89**
Body as a Whole	**80**	**12**
Asthenia	43	3
Infection	29	5
Chills	24	<1
Fever	17	1
Abdominal Pain	16	3
Pain	13	1
Headache	12	1
Throat Irritation	10	0
Back Pain	8	1
Flushing	6	0
Cardiovascular System	**17**	**3**
Hypotension	6	1
Digestive System	**48**	**3**
Nausea	31	1
Vomiting	12	0
Diarrhea	9	<1
Anorexia	8	0
Abdominal Enlargement	5	0
Constipation	5	0
Hemic and Lymphatic System	**98**	**86**
Thrombocytopenia	95	63
Neutropenia	77	60
Anemia	61	17
Ecchymosis	7	<1
Metabolic and Nutritional Disorders	**23**	**3**
Peripheral Edema	8	1
Angioedema	5	<1
Musculoskeletal System	**18**	**1**
Arthralgia	7	1
Myalgia	7	<1
Nervous System	**27**	**2**
Dizziness	10	<1
Insomnia	5	0
Respiratory System	**36**	**3**
Dyspnea	14	2
Increased Cough	10	0
Rhinitis	6	0
Bronchospasm	5	0
Skin and Appendages	**28**	**1**
Pruritus	9	<1
Rash	8	<1
Special Senses	**7**	**<1**
Urogenital System	**6**	**<1**

[†]Adverse reactions were captured for a period of 12 weeks following the first rituximab infusion of the Zevalin therapeutic regimen

The following adverse reactions (except for those noted in Table 2) occurred in between 1 and 4% of patients: urticaria (4%), anxiety (4%), dyspepsia (4%), sweats (4%), petechiae (3%), epistaxis (3%), allergic reaction (2%), and melena (2%).

Severe or life-threatening adverse reactions occurring in 1-5% of patients included pancytopenia (2%), infusion reaction (1%), gastrointestinal hemorrhage (1%), melena (1%), tumor pain (1%), and apnea (1%). The following severe or life-threatening reactions occurred in <1% of patients: angioedema, tachycardia, urticaria, arthritis, lung edema, pulmonary embolus, encephalopathy, hematemesis, subdural hematoma, and vaginal hemorrhage.

Hematologic Reactions
Hematologic toxicity was the most frequently observed adverse reaction in clinical trials. Patients in clinical studies were not permitted to receive hematopoietic growth factors beginning 2 weeks prior to administration of the Zevalin therapeutic regimen. Table 3 presents the incidence and duration of severe hematologic toxicity for patients with normal baseline platelet count

(≥ 150,000/mm^3) treated with the Zevalin therapeutic regimen and patients with mild thrombocytopenia (platelet count 100,000 to 149,000/mm^3) at baseline who were treated with a modified Zevalin therapeutic regimen that included a lower Y-90 Zevalin dose at 0.3 mCi per kg (11.1 MBq per kg).

Severe Hematologic Toxicity

Baseline Platelet Count	**≥ 150,000mm^3**	**100,000 to 149,000/mm^3**
Y-90 Zevalin Dose	**0.4 mCi/kg (14.8 MBq/kg)**	**0.3 mCi/kg (11.1 MBq/kg)**
ANC		
Median nadir (per mm^3)	800	600
Per Patient Incidence ANC <1000/mm^3	57%	74%
Per Patient Incidence ANC <500/mm^3	30%	35%
Median Duration (Days)* ANC <1000/mm^3	22	29
Platelets		
Median nadir (per mm^3)	41,000	24,000
Per Patient Incidence Platelets <50,000/mm^3	61%	78%
Per Patient Incidence Platelets <10,000/mm^3	10%	14%
Median Duration (Days)# Platelets <50,000/mm^3	24	35

*Day from last ANC ≥1000/mm^3 to first ANC ≥1000/mm^3 following nadir, censored at next treatment or death
Day from last platelet count ≥50,000/mm^3 to day of first platelet count ≥50,000/mm^3 following nadir, censored at next treatment or death

Median time to ANC nadir was 62 days, to platelet nadir was 53 days, and to hemoglobin nadir was 68 days. Information on growth factor use and platelet transfusions is based on 211 patients for whom data were collected. Filgrastim was given to 13% of patients and erythropoietin to 8%. Platelet transfusions were given to 22% of patients and red blood cell transfusions to 20%.

Infections
During the first 3 months after initiating the Zevalin therapeutic regimen, 29% of patients developed infections. Three percent of patients developed serious infections comprising urinary tract infection, febrile neutropenia, sepsis, pneumonia, cellulitis, colitis, diarrhea, osteomyelitis, and upper respiratory tract infection. Life threatening infections were reported for 2% of patients that included sepsis, empyema, pneumonia, febrile neutropenia, fever, and biliary stent-associated cholangitis. During follow-up from 3 months to 4 years after the start of treatment with Zevalin, 6% of patients developed infections. Two percent of patients had serious infections comprising urinary tract infection, bacterial or viral pneumonia, febrile neutropenia, perihilar infiltrate, pericarditis, and intravenous drug-associated viral hepatitis. One percent of patients had life threatening infections that included bacterial pneumonia, respiratory disease, and sepsis.

Secondary Leukemia and Myelodysplastic Syndrome
There were 19 cases of MDS/AML reported among 746 (2.6%) patients included in clinical studies and the expanded access programs, with a median follow-up of 4.4 years. The overall incidence of MDS/AML among the 211 patients included in the clinical studies was 5.2% (11/211), with a median follow-up of 6.5 years and median time to development of MDS/AML of 2.9 years. The cumulative Kaplan-Meier estimated incidence of MDS/secondary leukemia in this patient population was 2.2% at 2 years and 5.9% at 5 years. The incidence of MDS/AML among the 535 patients in the expanded access programs was 1.5% (8/535) with a median follow-up of 4.4 years and median time to development of MDS/AML of 1.5 years. Multiple cytogenetic abnormalities were described, most commonly involving chromosomes 5 and/or 7. The risk of MDS/AML was not associated with the number of prior treatments (0-1 versus 2-10).

7.2 Adverse Event Definitions and Characteristics

- **Adverse event (AE) or experience:** An adverse event is any unfavorable sign, symptom, or disease temporally associated with the use of study therapy, whether or not considered related to the study therapy.

- **Adverse drug reaction (ADR):** Any unexpected or dangerous reaction to a drug or an unwanted effect caused by the administration of a drug.

- **Serious adverse event or experience (SAE):** any AE/ADR occurring at any dose that results in any of the following outcomes:

  (a) Death;
  (b) A life-threatening AE/ADR (i.e., the patient/subject was, in the view of the initial reporter/investigator, at immediate risk of death from the AE/ADR as it occurred. It does not refer to an AE/ADR that hypothetically might have caused death if more severe);
  (c) Inpatient hospitalization or prolongation of existing hospitalization (Any adverse event requiring in-patient hospitalization i.e, hospitalization was required to treat or diagnose the AE/ADR, regardless of duration. A visit to the emergency room or hospital clinic would not be considered inpatient hospitalization; however, it could be considered serious if medical intervention was necessary in order to prevent a serious outcome);
  (d) A persistent or significant disability or incapacity (disability here means that there is a substantial disruption of a person's ability to conduct normal life functions);
  (e) A congenital anomaly/birth defect.
  (f) An important medical event (i.e., AEs/ADRs that might not be immediately life-threatening, or result in death or hospitalization might be considered serious when, based upon appropriate medical and scientific judgment, they might jeopardize the patient/subject or might require medical or surgical intervention to prevent one of the other serious outcomes listed above);
  (g) Any suspected transmission via a medicinal product of an infectious agent;

- **CTCAE term (AE description) and grade:** The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 will be utilized for AE reporting. All appropriate treatment areas should have access to a copy of the CTCAE version 4.0. A copy of the CTCAE version 4.0 can be downloaded from the CTEP web site (http://ctep.cancer.gov).

- **'Expectedness'**: AEs can be 'Unexpected' or 'Expected' (see Section 7.1 above) for expedited reporting purposes only. 'Expected' AEs (the ASAEL) are ***bold and italicized*** in the CAEPR (Section 7.1.1).

- **Attribution** of the AE:
  - Definite – The AE *is clearly related* to the study treatment.
  - Probable – The AE *is likely related* to the study treatment.
  - Possible – The AE *may be related* to the study treatment.
  - Unlikely – The AE *is doubtfully related* to the study treatment.
  - Unrelated – The AE *is clearly NOT related* to the study treatment.

7.3 Expedited Adverse Event Reporting

Any serious adverse events which occur once the first dose of treatment has been administered or within 30 days of receiving the last dose of study medication, whether or not related to the study drug, must be reported by the investigator. In addition, any SAEs which occur as a result of protocol specific diagnostic procedures or interventions must also be reported.

All serious adverse events as defined in 21 CFR 314.80 (whether initial or follow-up) must be reported to the IRB at local institution and the Project Manager at Dartmouth-Hitchcock Medical Center. Please send the SAE report by facsimile to Dartmouth-Hitchcock Medical Center (603-650-7799) or email the project manager within 1 business day of becoming aware of the SAE.

As the sponsor, Dartmouth-Hitchcock Medical Center will report to the Dartmouth College Institutional Review Board (Committee for the Protection of Human Subjects), Cephalon (610-738-6396) and Spectrum Pharmaceuticals (617-679-2979) using the SAE Transmittal Forms provided by Cephalon and Spectrum, respectively.

For the initial report to Spectrum, the Project Manager at DHMC will complete the provided Biogen Idec Adverse Event reporting form DSRM-F-18 v1.0 and fax to 617-679-2979 with all relevant documentation (laboratory tests, hospital admission/discharge summary, etc.) Please assure that participant identifiers are redacted from all documentation, except for participant study ID#, initials and date of birth.

The initial SAE report must be reported within one business day of becoming aware of the SAE and comprise a full written summary detailing relevant aspects of the adverse events in question. Where applicable, information from relevant hospital case records and autopsy reports should be included. Follow-up information should be forwarded to Dartmouth-Hitchcock Medical Center as soon as available. DHMC will send follow-up reports to Cephalon and Spectrum Pharmaceuticals.

Instances of death or congenital abnormality, if brought to the attention of the investigator, **AT ANY TIME** after cessation of study medication, and linked by the investigator to a previous clinical trial, should be reported immediately to appropriate regulatory agencies.

The Principal Investigator shall use his/her judgment to determine the relationship between the Serious Adverse Drug Experience and the Study Drug.

The institution shall notify the IRB and Cephalon and Spectrum within one (1) business day, by facsimile, upon learning of the occurrence during the Study of:

(a) All Serious AE/ADR as defined in Section 7.2, regardless of causality;
(b) All Expedited AE/ADR of Interest as described in 7.3.1
(c) Any exposure of a pregnant Study participant to the Study Drug within thirty

(30) days of exposure;

(d) A female partner of a male study participant becoming pregnant within thirty (30) days of exposure;

(e) Any medical event which may reasonably be believed to impair the integrity, validity or ongoing viability of the Study.

All such occurrences listed in this section shall be reported to Cephalon and Spectrum Pharmaceuticals using the SAE Transmittal Form provided by Cephalon and Spectrum Pharmaceuticals. In the event the IRB requests additional safety information from the Sponsor-Investigator, the Sponsor-Investigator shall notify Cephalon and Spectrum of such requests within one (1) business day of receiving the IRB request.

7.4 Safety and Data Monitoring

The Norris Cotton Cancer Center's Safety and Data Monitoring Committee (SDMC) will monitor the study progress on a quarterly basis according to the Cancer Center's guidelines. (See Appendix C).

7.5 On-Site Monitoring

Representatives of Dartmouth-Hitchcock Medical Center must be allowed to visit all study site locations periodically to assess the regulatory compliance, data quality and study integrity. On site they will review study records and directly compare them with source documents, discuss the conduct of the study with the investigator, and verify that the facilities remain acceptable. They may request access to all regulatory documents, source documents, CRFs, and other study documentation for on-site inspection. Direct access to these documents must be guaranteed by the investigator, who must provide support at all times for these activities.

7.6 Investigator and Site Responsibility for Drug Accountability

Accountability for the study drug at the trial site is the responsibility of the principal investigator at each site. Drug accountability records indicating the drug’s delivery date to the site, inventory at the site, use by each subject, or disposal of the drug, if approved by the principal investigator, will be maintained by the clinical site. Dartmouth-Hitchcock or its designee will review drug accountability at the site on an ongoing basis. All material containing study drug will be treated and disposed of as hazardous waste in accordance with governing regulations.

7.7 Investigator Compliance

The study shall be conducted as described in this approved protocol. All revisions to the protocol must be discussed with, and be prepared by, Dartmouth-Hitchcock. The investigator must not implement any deviation or change to the protocol without prior review and documented approval/favorable opinion from the IRB of an amendment, except where necessary to eliminate an immediate hazard(s) to study participants. Any significant deviation must be reported to the coordinating site, reported to the IRB if it meets their guidelines for reporting, and documented in the CRF.

When immediate deviation from the protocol is required to eliminate an immediate hazard(s) to subjects, the investigator will contact Dartmouth-Hitchcock, or a designee, if circumstances permit, to discuss the planned course of action. Any departures from the protocol must be documented. If the deviation meets guidelines for reporting, it must be reported to the institutional IRB, as well as the Committee for the Protection of Human Subjects at Dartmouth. The deviation form used for each institution can be submitted to the Project Manager at Dartmouth-Hitchcock Medical Center.

8. PHARMACEUTICAL INFORMATION

A list of the adverse events and potential risks associated with the investigational or commercial agents administered in this study can be found in Section 7.1.

8.1 Bendamustine (please see Treanda® package insert for more details)

Availability
Bendamustine is an investigational agent for the first line treatment of follicular lymphoma, and it is supplied to investigators by Cephalon, Inc.

FDA approved indications
Bendamustine is indicated for the treatment of patients with chronic lymphocytic leukemia, and for the treatment of patients with indolent B-cell non-Hodgkin's lymphoma that has progressed during or within six months of treatment with rituximab or a rituximab-containing regimen.

Description
Bendamustine contains bendamustine hydrochloride, an alkylating drug, as the active ingredient. The chemical name of bendamustine hydrochloride is 1H-benzimidazole-2-butanoic acid, 5-[bis(2-chloroethyl)amino]-1 methyl-, monohydrochloride. Its empirical molecular formula is C16H21Cl2N3O2 · HCl, and the molecular weight is 394.7. Bendamustine hydrochloride contains a mechlorethamine group and a benzimidazole heterocyclic ring with a butyric acid substituent.

Bendamustine is intended for intravenous infusion only after reconstitution with 20 mL of Sterile Water for Injection, USP, and after further dilution with either 0.9% Sodium Chloride Injection, USP, or 2.5% Dextrose/0.45% Sodium Chloride Injection, USP. It is supplied as a sterile non-pyrogenic white to off-white lyophilized powder in a single-use vial. Each vial contains 100 mg of bendamustine hydrochloride and 170 mg of mannitol, USP. The pH of the reconstituted solution is 2.5 - 3.5.

Mechanism of Action
Bendamustine is a bifunctional mechlorethamine derivative containing a purine-like benzimidazole ring. Mechlorethamine and its derivatives form electrophilic alkyl groups. These groups form covalent bonds with electron-rich nucleophilic moieties, resulting in interstrand DNA crosslinks. The bifunctional covalent linkage can lead to cell death via several pathways. Bendamustine is active against both quiescent and dividing cells. The exact mechanism of action of bendamustine remains unknown.

Pharmacokinetics
Absorption
Following a single IV dose of bendamustine hydrochloride Cmax typically occurred at the end of infusion. The dose proportionality of bendamustine has not been studied.

Distribution
In vitro, the binding of bendamustine to human serum plasma proteins ranged from 94-96% and was concentration independent from 1-50 μg/mL. Data suggest that bendamustine is not likely to displace or to be displaced by highly protein-bound drugs. The blood to plasma

concentration ratios in human blood ranged from 0.84 to 0.86 over a concentration range of 10 to 100 μg/mL indicating that bendamustine distributes freely in human red blood cells. In humans, the mean steady state volume of distribution (Vss) was approximately 25 L.

Metabolism
In vitro data indicate that bendamustine is primarily metabolized via hydrolysis to metabolites with low cytotoxic activity. *In vitro*, studies indicate that two active minor metabolites, M3 and M4, are primarily formed via CYP1A2. However, concentrations of these metabolites in plasma are 1/10 and 1/100 that of the parent compound, respectively, suggesting that the cytotoxic activity is primarily due to bendamustine. *In vitro* studies using human liver microsomes indicate that bendamustine does not inhibit CYP1A2, 2C9/10, 2D6, 2E1, or 3A4/5. Bendamustine did not induce metabolism of CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2E1, or CYP3A4/5 enzymes in primary cultures of human hepatocytes.

Elimination
No mass balance study has been undertaken in humans. Preclinical radiolabeled bendamustine studies showed that approximately 90% of drug administered was recovered in excreta primarily in the feces. Bendamustine clearance in humans is approximately 700 mL/minute. After a single dose of 120 mg/m2 bendamustine IV over 1-hour the intermediate t½ of the parent compound is approximately 40 minutes. The mean apparent terminal elimination t½ of M3 and M4 are approximately 3 hours and 30 minutes respectively. Little or no accumulation in plasma is expected for bendamustine administered on Days 1 and 2 of a 28-day cycle.

Renal Impairment
In a population pharmacokinetic analysis of bendamustine in patients receiving 120 mg/m2 there was no meaningful effect of renal impairment (CrCL 40 - 80 mL/min, N=31) on the pharmacokinetics of bendamustine. Bendamustine has not been studied in patients with CrCL < 40 mL/min. These results are however limited, and therefore bendamustine should be used with caution in patients with mild or moderate renal impairment. Bendamustine should not be used in patients with CrCL < 40 mL/min.

Hepatic Impairment
In a population pharmacokinetic analysis of bendamustine in patients receiving 120 mg/m^2 there was no meaningful effect of mild (total bilirubin ≤ ULN, AST ≥ ULN to 2.5 x ULN, and/or ALP ≥ ULN to 5.0 x ULN, N=26) hepatic impairment on the pharmacokinetics of bendamustine. Bendamustine has not been studied in patients with moderate or severe hepatic impairment. These results are however limited, and therefore bendamustine should be used with caution in patients with mild hepatic impairment. Bendamustine should not be used in patients with moderate (AST or ALT 2.5 - 10 x ULN and total bilirubin 1.5 - 3 x ULN) or severe (total bilirubin > 3 x ULN) hepatic impairment.

Effect of Age
Bendamustine exposure (as measured by AUC and Cmax) has been studied in patients ages 31 through 84 years. The pharmacokinetics of bendamustine (AUC and Cmax) were not significantly different between patients less than or greater than/equal to 65 years of age.

Effect of Gender
The pharmacokinetics of bendamustine were similar in male and female patients.

Effect of Race
The effect of race on the safety, and/or efficacy of bendamustine has not been established. Based on a cross-study comparison, Japanese subjects (n = 6) had on average exposures that were 40% higher than non-Japanese subjects receiving the same dose. The significance of this difference on the safety and efficacy of bendamustine in Japanese subjects has not been established.

Pharmacokinetics/Pharmacodynamics
Based on the pharmacokinetics/pharmacodynamics analyses of data from NHL patients, a correlation was observed between nausea and bendamustine Cmax.

8.2 **90-yttrium (Y) ibritumomab tiuxetan**

Availability
90-yttrium (Y) ibritumomab tiuxetan is commercially available. It is supplied to investigators through coverage by the patients' insurance.

FDA indications
90-yttrium (Y) ibritumomab tiuxetan (Zevalin) is indicated for the treatment of patients with relapsed or refractory, low-grade or follicular B-cell non-Hodgkin's lymphoma (NHL), including patients with rituximab refractory follicular NHL. It is in the NCCN compendium for the first-line treatment of follicular lymphoma. On 9/5/2009 the FDA expanded the label to include patients with previously untreated follicular non-Hodgkin's Lymphoma (NHL), who achieve a partial or complete response to first-line chemotherapy.

Mechanism of Action
Ibritumomab tiuxetan binds specifically to the CD20 antigen (human B-lymphocyte-restricted differentiation antigen, Bp35). The apparent affinity (KD) of ibritumomab tiuxetan for the CD20 antigen ranges between approximately 14 to 18 nM. The CD20 antigen is expressed on pre-B and mature B lymphocytes and on > 90% of B-cell non-Hodgkin's lymphomas (NHL). The CD20 antigen is not shed from the cell surface and does not internalize upon antibody binding. The chelate tiuxetan, which tightly binds In-111 or Y-90, is covalently linked to ibritumomab. The beta emission from Y-90 induces cellular damage by the formation of free radicals in the target and neighboring cells. Ibritumomab tiuxetan binding was observed *in vitro* on lymphoid cells of the bone marrow, lymph node, thymus, red and white pulp of the spleen, and lymphoid follicles of the tonsil, as well as lymphoid nodules of other organs, such as the large and small intestines.

Pharmacodynamics
In clinical studies, administration of the Zevalin therapeutic regimen resulted in sustained depletion of circulating B cells. At four weeks, the median number of circulating B cells was zero (range, 0-1084/mm3). B-cell recovery began at approximately 12 weeks following treatment, and the median level of B cells was within the normal range (32 to 341/mm3) by 9 months after treatment. Median serum levels of IgG and IgA remained within the normal range throughout the period of B-cell depletion. Median IgM serum levels

dropped below normal (median 49 mg/dL, range 13-3990 mg/dL) after treatment and recovered to normal values by 6-months post therapy.

Pharmacokinetics
Pharmacokinetic and biodistribution studies were performed using In-111 Zevalin (5 mCi [185 MBq] In-111, 1.6 mg ibritumomab tiuxetan). In an early study designed to assess the need for pre-administration of unlabeled antibody, only 18% of known sites of disease were imaged when In-111 Zevalin was administered without unlabeled ibritumomab. When preceded by unlabeled ibritumomab (1.0 mg/kg or 2.5 mg/kg), In-111 Zevalin detected 56% and 92% of known disease sites, respectively. These studies were conducted with a Zevalin therapeutic regimen that included unlabeled ibritumomab.

In pharmacokinetic studies of patients receiving the Zevalin therapeutic regimen, the mean effective half-life for Y-90 activity in blood was 30 hours, and the mean area under the fraction of injected activity (FIA) vs. time curve in blood was 39 hours. Over 7 days, a median of 7.2% of the injected activity was excreted in urine.

8.3 Rituximab (refer to RITUXAN package insert for more information)

Rituximab (Rituxan®) is a genetically engineered chimeric murine/human monoclonal antibody directed against the CD20 antigen found on the surface of normal and malignant B lymphocytes.

It is indicated for the treatment of patients with relapsed or refractory, low-grade or follicular, CD 20-positive B-cell non-Hodgkin’s lymphoma.

Rituximab is commercially available.

Rituximab should be administered per the guidelines found in the package insert.

9. CORRELATIVE/SPECIAL STUDIES

9.1 Qualitative PCR for BCL2 translocation

A bone marrow aspirate and biopsy, as well as a peripheral blood sample will be taken:

- At screening
- 4-6 weeks after the last cycle of bendamustine and rituximab
- 12-16 weeks after 90-yttrium (Y) ibritumomab tiuxetan

In order to standardize results, these research samples will be analyzed by polymerase chain reaction (PCR) for the bcl-2/JH t(14;18) translocation using a qualitative PCR assay at the Dartmouth-Hitchcock Department of Pathology – Molecular Pathology (by Gregory Tsongalis, Ph.D.)

Correlative science kits will be provided to participating institutions and will include:
1. EDTA anticoagulated vacutainer (3-5 mL whole blood)
2. EDTA anticoagulated vacutainer (1-3 mL bone marrow)
3. Forms (Appendix A) to be filled out indicating patient registration number, date and time sample taken, time point taken indicating “baseline”, “post-B-R”, or “post-RIT”, participating institution, responsible MD, and responsible local study coordinator.

The bone marrow aspirate (lavender top) and peripheral blood (pink top) in EDTA tubes should be shipped the same day (Monday through Thursday) at room temperature (ambient). The lab cannot accept samples over the weekend.

The specimens will be shipped via overnight courier (Mon-Thurs) to:

Molecular Pathology Laboratory
Room 483S01
Borwell 4 East
Dartmouth Hitchcock Medical Center
1 Medical Center Drive
Lebanon, NH 03756

Tel# 603-650-8257

In the event that a bone marrow aspirate was already performed prior to screening, DHMC will accept a frozen bone marrow aspirate, or non-decalcified paraffin embedded biopsy if completed within 30 days of screening. The aspirate must be frozen at -20 degrees Celsius. The sample can then be shipped to DHMC on dry ice. The non-decalcified paraffin embedded biopsy can be shipped at room temperature.

9.2 Genetic Polymorphisms

Within the context of this clinical trial, we plan to study specific genetic polymorphisms that have been known to correlate with clinical and molecular response to rituximab therapy, including polymorphisms in FcgammaRIIIa and FcgammaRIIa. We hope to gain insight into molecular predictors of rituximab sensitivity and resistance. We also plan to learn about factors related to bendamustine sensitivity and resistance. Additionally, we will study if there

are polymorphisms or mutations of the CD20 protein that correlate with radioimmunotherapy sensitivity or resistance. See Appendix D for a detailed protocol on this correlative study. We plan to use separate research funds to do this specific correlative research.

10. STUDY CALENDAR

*Pre-Study evaluations are to be conducted within 2 weeks prior to start of treatment. CT scans can be done within 4 weeks prior to the start of treatment.
^Each cycle of B-R is 28 days (±2 days) for 4 cycles

	Pre-Study*	**BR^** Cycle 1 Day -7 (±1 day)[k]	**B-R^** Day 1 Cycles 1-4	**B-R^** Day 2 Cycles 1-4	4-6 weeks[e] after **B-R**	**RIT** Day 1 (6-12 weeks[f] after B-R)	**RIT** Day 8 (±1 day)	Every 4 weeks after RIT until count recovery[h]	**End of Treatment** 12-16 weeks[e] after RIT[g]	**Follow-Up[g]**
Rituximab 375mg/m2		X	X							
Bendamustine			X	X						
Rituximab 250mg/m2						X	X			
90-(Y) ibritumomab tiuxetan							X			
Informed consent	X									
Demographics	X									
Medical history and FLIPI 1 and 2 score	X									
Concurrent meds	X	X	X		X	X		X	X	
Physical exam	X	X	X		X	X		X	X	X
Vital signs (Heart rate, blood pressure, temperature)	X	X	X	X	X	X		X	X	X
Height	X									
Weight	X	X	X		X	X		X	X	X
ECOG Performance status	X	X	X		X	X		X	X	X
CBC w/diff, plts[a]	X	X	X		X	X	X	X[i]	X	X
Serum chemistry[a]	X	X	X		X	X		X	X	X[l]
B2 Microglobulin	X									
Adverse event evaluation		X	X	X	X	X	X	X	X	
CT Scan[b]	X				X				X	X
B-HCG[c]	X					X				
Bone Marrow Aspirate and Biopsy[j]	X				X[d]				X	
BCL2 evaluation (BM aspirate and peripheral blood)	X				X[d]				X	

a. **Serum chemistry requirements are Sodium, Potassium, Chloride, Bicarbonate, BUN, Creatinine, Magnesium, Phosphorus, Calcium, Total Protein, Albumin, Total Bilirubin, AST, ALT, LDH, uric acid. All labs are to be checked prior to the initiation of each cycle. Lab monitoring in between cycles of B-R will be left to the discretion of the investigator.**

b. **The pre-study CT scan must be conducted within 4 weeks of starting treatment. CT scans to measure target lesions will occur pre-study, 4-6 weeks after completion of B-R, and 12-16 weeks after 90-(Y) ibritumomab tiuxetan. After this point, CT scans must be performed every 6 months for the first 2 years, then annually. If the patient does not complete treatment per protocol, an EOT evaluation should occur within 28 days that the decision was made to take the patient off treatment. .**

c. **For females of childbearing potential, a urine or serum B-HCG should be done at screening and within 1 week of the first dose of 90-(Y) ibritumomab tiuxetan.**

d. **The bone marrow aspirate and biopsy should occur 4-6 weeks after the last dose of B-R. The research sample for both bone marrow aspirate and peripheral blood should be taken at this time. If a participant is not eligible to receive 90-yttrium Ibritumomab Tiuxetan, then a bone marrow biopsy does not need to be repeated at EOT.**

e. **Although a maximum of 6 and 16 weeks are allowed for the restaging visits, respectively, every effort should be made to restage as close to 4 and 12 weeks, respectively.**

f. **Although a maximum of 12 weeks is allowed to begin RIT, every effort should be made to begin RIT as close to 6 weeks after the last cycle of B-R as possible**

g. **All participants will be followed after EOT every 3 months (+ 2 weeks) for 2 years and then annually for a maximum of 10 years from study entry or death, whichever occurs first.**

h. **Count recovery following RIT is defined as ANC ≥1000/mm^3 and platelet count ≥100,000/mm^3**

i. **CBC w/diff, plts will be monitored WEEKLY following RIT until count recovery. These weekly labs can be drawn at a local facility for participant convenience if necessary.**

j. **A bone marrow aspirate, or non-decalcified paraffin embedded biopsy will be accepted if done within 30 days of screening.**

k. **Prestudy evaluation and procedures done within 7 days of C1D-7 are acceptable to be used for C1D-7 and do not need to be repeated.**

l. **Effective October 19, 2016 – magnesium, phosphorus, and uric acid are no longer required during follow-up.**

11. MEASUREMENT OF EFFECT

11.1 Assessment of Response

The International Response Criteria for non-Hodgkin's Lymphoma will be used to assess response. (Cheson, JCO Vol 17, Issue 4 (April), 1999: 1244). **CT scans will be used to determine the size of the target lesions. PET scans may be used at the discretion of the investigator, but should not be used as a replacement for CT scan.**

For the purposes of this study, patients should be evaluated by CT scan at baseline, after the completion of the last cycle of B-R, and 12-16 weeks after receiving 90-(Y) ibritumomab tiuxetan. Follow up CT scans will occur every 6 months for 2 years, then annually. Any additional radiographic studies may be performed at the discretion of the investigator. Bi-dimensional nodal measurements for lesions must be recorded on the CT report. A maximum of 6 lesions should be selected that are measurable in at least 2 perpendicular dimensions, representative of all involved organs/areas, suitable for accurate repeat measurements, and include mediastinal and retroperitoneal areas of disease.
To define response, select sites and measure the two longest diameters. Multiply each node's two longest diameters together, then Sum the Products of the greatest transverse Diameters (SPD). Calculate the percent increase or decrease. For percent decrease, calculate (baseline-current disease assessment)/baseline. For percent increase, calculate (current disease assessment-smallest sum)/smallest sum. This will allow for accurate calculations to define response (see Appendix G)
For this study, a CR and CRu will be categorized as CR for statistical analyses.

11.2 Definitions

Response Criteria for Non-Hodgkin's Lymphoma

Response Category	Physical Examination	Lymph Nodes	Lymph Node Masses	Bone Marrow
CR	Normal	Normal	Normal	Normal
CRu	Normal	Normal	Normal	Indeterminate
	Normal	Normal	> 75% decrease	Normal or indeterminate
PR	Normal	Normal	Normal	Positive
	Normal	≥50% decrease	≥50% decrease	Irrelevant
	Decrease in liver/spleen	≥50% decrease	≥50% decrease	Irrelevant
Relapse/progression	Enlarging liver/spleen; new sites	New or increased	New or increased	Reappearance

Progression-Free Survival (PFS)
PFS is defined as the duration of time from start of treatment to time of progression or death from any cause whichever comes first.

Time to next treatment (TTNT)
TTNT is defined as the duration of time from end of study treatment (Y-90 ibritumomab tiuxetan) to next treatment either due to relapse or progression of disease, or death due to any cause, whichever comes first.

11.3 **Methods for Evaluation of Measurable Disease**

All measurements should be taken and recorded in metric notation using a ruler or calipers. All screening evaluations should be performed within 2 weeks of beginning of treatment, excluding CT scans which can be performed within 4 weeks of beginning treatment.

The same method of assessment and the same technique should be used to characterize each identified and reported lesion at baseline and during follow-up. Imaging-based evaluation is preferred to evaluation by clinical examination when both methods have been used to assess the antitumor effect of a treatment.

Clinical lesions Clinical lesions will only be considered measurable when they are superficial (e.g., skin nodules and palpable lymph nodes). In the case of skin lesions, documentation by color photography, including a ruler to estimate the size of the lesion, is recommended.

CT is the preferred radiographic modality. PET scans can be performed in addition to CT at the discretion of the investigator.

Conventional CT. This technique should be performed per institutional guidelines. Cuts of 10 mm or less in slice thickness contiguously are recommended but not required. It is also recommended that spiral CT should be performed using a 5 mm contiguous reconstruction algorithm. This applies to tumors of the chest, abdomen, and pelvis. Head and neck tumors and those of extremities may be done as per institutional guidelines.

12. STATISTICAL CONSIDERATIONS

12.1 Study Design/Endpoints

The primary endpoint is complete response (CR) rate. Historical complete response (CR) rate has been 35%. This rate will be considered as the null hypothesis. The alternative hypothesis is that bendamustine-rituximab (B-R) followed by radioimmunotherapy (RIT) will increase the CR rate to 55%. An optimal Simon two-stage design will be incorporated to allow an early futility look during the Phase II component while minimizing the expected total sample size. It is calculated to be 14 in the first stage. If more than 5 subjects have a CR then an additional stage of 25 patients will be added for a total of 39. The probability of early termination is 64% under the null hypothesis and 12% under the alternative. The expected sample size (i.e., average if this study was repeated many times) is 24.8. These calculations assume a type I error rate of 5% and power of 80%.

The CR rate will calculated by CR + CRu divided by the # of evaluable subjects x 100%.

The ORR will be calculated by CR + CRu + PR divided by the # of evaluable subjects x 100%.

Statistical analysis for the primary endpoint will include two pre-planned analyses: The first analysis will include only participants who complete all protocol therapy (4 cycles of bendamustine and rituximab followed by Y-90 ibritumomab tiuxetan). If a participant does not complete all study therapy, he/she will be replaced with another participant in a 1 to 1 fashion until there are a total of 39 evaluable participants. Since the statistics for the primary endpoint are based around additive sequential treatment, we will be performing this analysis.

The second analysis will include all participants who receive any portion of study therapy (intention-to-treat analysis).

Both statistical analyses will be reported.

12.2 Sample Size/Accrual Rate

39 evaluable participants. At the accrual rate of about 2 participants per month this trial should accrue in 20 months, and be completed in 28 months. Reporting of response rate would be possible at 31 months. At the accrual rate of 4 participants per month, this trial should accrue in 10 months, and be completed in 18 months. Reporting of response rate would be possible at 21 months. With an expected dropout rate of 5 to 10%, 41 to 44 participants will need to be accrued to this study to reach 39 evaluable participants.

All participants will be evaluable for toxicity.

12.3 Analysis of Secondary Endpoints

The secondary endpoints are safety of this combination. The CTCAE v4.0 will be used to determine toxicities.

In addition we will calculate the CR and OR rate after B-R and Y-90 ibritumomab tiuxetan. The conversions from PR to CR will be reported.

The progression-free survival and time to next treatment of all participants treated will be determined with adequate follow-up.

12.4 **Analysis of Exploratory Endpoints**

Molecular Correlations: The association of molecular Bcl-2 detection by PCR with CR and OR will be analyzed using Cox's model with time-dependent covariates, as Bcl-2 is a longitudinal variable measured at three time-points. The hazard of complete, or partial response, the hazard of mortality, the hazard of progression or death, or time to next treatment or death, will be modeled based on longitudinal Bcl-2, and Wald test reported. In addition we shall use a joint longitudinal-survival model to characterize these associations.

APPENDIX A

Correlative Science Blood and Marrow Samples Form

ENCLOSE THIS FORM WITH SPECIMENS

PATIENT IDENTIFICATION #: _____ ***INITIALS:*** _____

DATE SAMPLE TAKEN: _____

DATE SAMPLE SHIPPED: _____

Indicate: □ ***Peripheral Blood*** ***Time sample taken:*** _____
□ ***Bone Marrow*** ***Time sample taken:*** _____
□ ***Paraffin-embedded biopsy*** ***Time sample taken:*** _____

Indicate: □ ***"Baseline"***
□ ***"Post B-R"Date of Cycle 1 (Day 1) B-R*** _____

Date of last Cycle (Day 1) of B-R _____
□ ***"Post RIT"Date of RIT*** _____

Institution: _____

Responsible MD: _____

Responsible Study Coordinator: _____ ***FAX#:*** _____

Please fax this form prior to enclosing in shipment to Fax # 603-650-7799

Ship samples via overnight courier (Mon-Thurs) to:

Molecular Pathology Laboratory
Room 483S01
Borwell 4 East
Dartmouth Hitchcock Medical Center
1 Medical Center Drive
Lebanon, NH 03756
Tel# 603-650-8257

INTERNAL USE ONLY

Date Received: _____ ***Received by (Printed Name):*** _____

Confirmation of receipt sent to fax#: _____ **Date:** _____

APPENDIX B

Follicular Lymphoma International Prognostic Index (FLIPI)

The Follicular Lymphoma International Prognostic Index uses five adverse factors to identify three risk groups (low, intermediate, and high) to provide clear differentiation of long term prognosis.

FLIPI-1

Age greater than 60 years?
Ann Arbor Stage III or IV?
Serum LDH greater than ULN?
Number of nodal sites greater than 4?
Pre-transfusion hemoglobin less than 12 g/dl?

To calculate a FLIPI-1 score, answer "yes" or "no" to each question. Add up the number of "yes" responses. The total number of "yes" responses will be the FLIPI-1 score. A 0-1 score is "low risk" and OS at 10 years is estimated to be 70%. If the score is 2, this is characterized as "intermediate risk" and OS at 10 years is estimated to be 50%. If the score is 3 or greater, that is considered "high risk" and OS at 10 years is estimated to be 35%.

FLIPI-2

Beta 2 microglobulin greater than ULN?
Longest diameter of largest involved lymph node greater than 6 cm?
Bone marrow involvement?
Pre-transfusion hemoglobin less than 12 g/dl?
Age greater than 60 years?

To calculate a FLIPI-2 score, answer "yes" or "no" to each question. Add up the number of "yes" responses. The total number of "yes" responses will be the FLIPI-2 score. A 0 score is "low risk" and PFS at 3 years is estimated to be 91%. If the score is 1-2, this is characterized as "intermediate risk" and PFS at 3 years is estimated to be 69%. If the score is 3 or greater, that is considered "high risk" and PFS at 3 years is estimated to be 51%.

APPENDIX C

Safety and Data Monitoring Committee (SDMC)

The Safety and Data Monitoring Committee (SDMC) is the Data and Safety Monitoring Board for the Norris Cotton Cancer Center at Dartmouth. It is a multidisciplinary committee charged with overseeing monitoring of safety of participants, conduct, progress, and validity and integrity of the data of all clinical trials at NCCC at Dartmouth. The committee is chaired by the Director of the Clinical Research Office, and meets quarterly to review the progress and safety of all active research protocol that are not reviewed by another safety and data monitoring committee. If a study is already being monitored by a safety and data monitoring committee formed by a national cooperative group, a pharmaceutical sponsor, or a study specific committee for a phase III trial, the NCCC SDMC does not actively monitor the study. The SDMC oversees the process of adverse event reporting to assure that the requirements are met. The SDMC has the authority to require amendments, suspend or terminate any research activities that fall within its jurisdiction. The SDMC reports to the Clinical Cancer Review Committee (CCRC). The SDMC can institute any other appropriate conditions needed for subject safety.

With the exception of the Office of Clinical Research (OCR) Regulatory and Compliance Officer who is a standing member, all members serve a five-year term. Committee membership and voting status are assigned by the Director of the NCCC. The Committee includes representatives from the following disciplines: medical oncology, hematology, radiation oncology, pharmacology, bio-statistics, ethics, oncology nursing and data management. Presence of three or more of the SDMC voting members constitutes a quorum. Members may be appointed on an ad hoc basis by the Chairman of the SDMC, should additional expertise be required in the review of certain studies.

The SDMC meets on a quarterly basis. Special meetings may be convened when necessary, for urgent concerns regarding patient safety or data integrity. Administration of the SDMC (paperwork, meeting coordination, minutes) is provided by the Office of Clinical Research. Minutes reflect the members present, substantive issues discussed, voting results, and members abstaining due to conflict of interest.

The frequency of SDMC monitoring for a particular study depends on the level of risk (low or high risk) assigned to the study by the Chairman of the CCRC, as described above. Low risk trials are reviewed on an annual basis by the SDMC. High risk trials are reviewed on a quarterly basis by the SDMC. The size and complexity of a trial also determines the frequency and level of review. Data and safety monitoring activities for each study continue until all patients have completed their treatment and all participants are beyond the time point at which study-related adverse events would likely be encountered. ***Since this is a multicenter study, this protocol will be reviewed on a quarterly basis.***

For each SDMC protocol review, summary information regarding toxicity and accrual patterns is prepared by the OCR staff, and submitted to the SDMC. The study Principal Investigator is required to review and sign all SDMC reports. Specific information submitted for review includes:

• The expected and actual numbers of participants entered to date, as well as for the most recent reporting period
• Number of participants treated

- Description of any changes to the study design since the last SDMC review
- Exceptions in eligibility or treatment
- Dates of participant enrollments
- (Dose tier for each participant, for Phase I studies)
- Best response to treatment for each participant, for Phase II and III studies
- (Treatment arm for each participant, for Phase III studies)
- The type and grade of adverse events for each participant using CTC 2.0 grading
- The duration and outcome of adverse events for each participant
- Current disease, vital, and study (on- or off-study) status of each participant
- Copies of all adverse event reports submitted to the IRB since the last SDMC review are also sent to the SDMC, so they can be reviewed for accuracy and timeliness
- Significant literature reporting developments that may affect the safety of participants or the ethics of the study
- Results of any interim analyses required by the protocol
- Copies of abstracts or papers written using study data

Members receive this information approximately one week prior to Committee meetings, to allow for preliminary study and review. Each study is assigned to a specific Committee member for presentation during the Committee meeting. In the event that a Committee member is either a principal investigator or co-principal investigator for a study under review, or has any other conflict of interest (including substantial financial interest in the study sponsor agency), that member may be present to answer questions regarding the study, but must abstain from voting and leave the room prior to final deliberations and voting on that study. In the event that the Chairman is the principal investigator for the study, another member of the Committee will oversee the Committee deliberations and voting.

The Committee may vote to take one of the following actions for each protocol reviewed:

- **Full Approval:** enrollment may continue; no outstanding questions regarding toxicity or accrual

- **Conditional Approval:** enrollment may continue conditional upon satisfactory response by the principal investigator to SDMC concerns regarding toxicities and/or accrual.

- **Suspension:** enrollment immediately suspended pending principal investigator response SDMC concerns regarding toxicity and/or accrual patterns.

- **Closure:** study closed due to unacceptable toxicity and/or accrual patterns.

All SDMC decisions are conveyed in writing to the study principal investigator. Principal investigators may appeal SDMC decisions in writing to the chairman of the SDMC. If the decision regarding the appeal is unsatisfactory to the investigator, a second appeal may be made to the CCRC.

APPENDIX D

Title: Polymorphisms associated with clinical and molecular responses in follicular lymphoma patients treated with rituximab and the novel agent bendamustine

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Abstract

Rituximab is a monoclonal antibody (mAb) directed against the CD20 antigen present on lymphoma cells. Rituximab has revolutionalized the treatment follicular lymphoma, showing significantly better response rates, progression-free survival and overall survival when added to chemotherapy. Despite these impressive outcomes, rituxmab's mechanisms of action are unknown. Rituximab works through many mechanisms, including antibody-dependent cellular cytotoxicity (ADCC), complement-dependent cytotoxicity (CDC), and direct apoptotic effects by signaling through CD20.

Polymorphisms of two IgG Fcγ receptors have been shown to predict clinical responses to rituximab therapy, presumably because of their role in ADCC. The correlation of polymorphisms and response in patients treated with rituximab combined with chemotherapy, or "chemoimmunotherapy" is unclear. The D1015 clinical trial is studying the clinical and molecular response rates of follicular lymphoma patients to rituximab and bendamustine. As a scientific correlative to this ongoing study, we will determine how polymorphisms in Fcγ receptors correlate with clinical and molecular responses. *We hypothesize that the response rates will be higher for patients with FcγRIIIA-158-V/V and FcγRIIa-131-H/H polymorphisms.* If this correlation is confirmed it adds further credence that rituximab primarily acts by way of ADCC. Until recently, R-CHOP has been the preferred first-line chemoimmunotherapy for the treatment of follicular lymphoma. The clinical trial D1015 uses a novel alkylator/purine analog hybrid, bendamustine, in combination with rituximab. This novel combination has shown superiority when compared to R-CHOP. *This study will be the first study of FcγR polymorphisms in follicular lymphoma patients treated with rituximab and the novel agent, bendamustine.*

Background

Rituximab is a chimeric IgG1 monoclonal antibody (mAb) directed against the B-cell surface antigen CD20 expressed on B-lymphocytes. Rituximab induces killing of normal and malignant B-cells expressing CD20. Rituximab's proposed mechanisms include: 1) elicitation of antibody-dependent cellular cytotoxicity (ADCC), 2) induction of lymphoma cell death through complement-dependent cytolysis (CDC) and/or complement-dependent cellular toxicity, and 3) direct induction of apoptosis following engagement of CD20 by rituximab.[21]

Clinical response to rituximab is dependent on specific FcγR polymorphisms. Cartron, et al. demonstrated that follicular lymphoma patients homozygous for FcγRIIIa-158-V/V [valine] had a significantly better clinical response rate (100%) compared to patients expressing heterozygous (V/F) or homozygous phenylalanine (F/F) genotype (67%).[22] In addition, Weng and Levy confirmed the FcγRIIIa-158-V/V association with clinical response in another cohort of follicular lymphoma patients.[23] They also reported that the polymorphism FcγRIIa-131-H/H [histidine] is independently associated with response rate compared to R [arginine] carriers.[23] Similarly, in a study of Waldenstrom's macroglobulinemia, another form of indolent lymphoma, response rates to rituximab correlated with FcγRIIIa-158 polymorphisms.[24] The response trend was higher for patients with FcγRIIIA-158-V/V, compared to heterozygous (V/F) or homozygous (F/F).[24] Thus, rituximab's major mechanism of action seems to be through ADCC.

In this model, the rituximab's Fc portion binds to Fcγ receptors on effector cells, including natural killer cells, and granulocytes. This binding leads to the destruction of rituximab-bound B-cells, either by phagocytosis or by the release of cytotoxic granules contained in the effector cells. Functional allelic polymorphisms of the three classes of FcγR (FcγRI, FcγRII, and FcγRIII) exist in humans and these generate different receptor properties affecting binding affinity for the different IgG subclasses including IgG1.[21] Genetic polymorphisms corresponding to phenotype expression of valine (V) or phenylalanine (F) at amino acid 158 on the FcγRIIIa greatly influences the affinity of IgG1 to the Fcγ receptor. Studies have demonstrated the stronger binding of IgG1 antibody to homozygous FcγRIIIa-158V/V NK cells than to homozygous FcγRIIIa-158F/F or heterozygous NK cells.[25, 26] Similarly, polymorphisms related to the expression of histidine (H) or arginine (R) at amino acid 131 in FcγRIIa affects the binding affinity of IgG1.[27] This evidence supports that rituximab, an IgG1 mAb, functions by ADCC due to higher affinity binding.

When rituximab is combined with chemotherapy, response rates improve significantly compared to chemotherapy or rituximab alone. The addition of rituximab to every chemotherapeutic combination studied (CVP, CHOP, FND, FCM, bendamustine)[1, 2, 4-6, 15, 17] has resulted in significant increase in the overall response rate and complete response rate. In addition, R-CVP has demonstrated a significant increase in overall survival when compared to CVP alone.[5] The mechanism of the added benefit of rituximab to chemotherapy is more elusive. For instance, in one study, when patients are sequentially treated with chemotherapy (CHOP) followed by rituximab, the association between FcγRIIIa and FcγRIIa is apparently lost.[28] However, in diffuse large B-cell lymphoma patients treated with R-CHOP, Kim, et al. demonstrated that the FcγRIIIa-158-V/V polymorphism was associated with responses and faster time to response.[29] Thus, it is still unclear whether rituximab works by ADCC in lymphoma patients treated with chemoimmunotherapy. It is also unclear whether rituximab acts differently when combined with chemotherapeutic agents other than CHOP, such as bendamustine. *This study will provide useful data as to the mechanism of rituximab's effect in follicular lymphoma patients treated with chemoimmunotherapy that incorporates the novel chemotherapeutic agent, bendamustine.* Bendamustine is a purine analog/alkylator hybrid cytotoxic chemotherapy with significant clinical activity in lymphoma. It is highly active in refractory indolent B-cell

lymphoma as a single agent with an overall response rate of 75%.[30] In front-line combination with rituximab compared to R-CHOP, bendamustine significantly improves overall response rate, complete response rate, and progression-free survival. Bendamustine and rituximab chemoimmunotherapy results in far fewer toxicities including less infectious complications, and practically no alopecia.[17]

Within the context of this clinical trial, we will examine specific genetic polymorphisms that correlate with clinical and molecular response the chemoimmunotherapy combination of bendamustine and rituximab. These results will provide additional knowledge addressing molecular predictors of rituximab sensitivity and resistance and will have important implications in personalizing immunotherapy for the treatment of follicular lymphoma.

Specific Aims

To determine the association of FcγRIIIa-158-V/V and FcγRIIa-131-H/H polymorphisms with clinical and molecular responses in follicular lymphoma patients treated with rituximab and bendamustine.

Methods

Patient samples will be collected and stored at study entry from consenting follicular lymphoma patients enrolled in D1015. In this proposed study we will examine FcγR (FcγRIIIa and FcγRIIa) polymorphisms known to correlate with response. In order to detect the FcγRIIIA polymorphisms, DNA will be extracted from peripheral blood mononuclear cells collected prior to treatment using a kit (Qiagen). Genotyping of FCGR3A-158V/F polymorphism will be performed as described by Cartron,[22] et al using a nested PCR followed by allele-specific restriction enzyme digestion. Briefly, 2 FCγRIIIA-specific primers (5'-ATATTTACAGAATGGCACAGG-3', 5'-GACTTGGTACCCAGGTTGAA-3') will be used to amplify a 1.2 kilobase fragment containing the polymorphic site. The initial PCR assay will be performed with 1.25µg genomic DNA, 200ng of each primer, 200µM of each deoxyribonucleoside triphosphate (dNTP) and 1U Taq DNApolymerase. This first PCR will consist of 10 minutes at 95°C, then 35 cycles (each consisting of steps at 95°C for 1 minute, 57°C for 1.5 minutes, and 72°C for 1.5 minutes), and 8 minutes at 72°C to achieve complete extension. The second PCR will use primers (5'-ATCAGATTCGATCCTACTTCTGCAGGGGGCAT-3', 5'-ACGTGCTGAGCTTGAGTGATGGTGATGTTCAC-3') amplifying a 94base pair (bp) fragment and creating an NlaIII restriction site only in the FCγRIIIa-158V allele. This nested PCR will be performed with 1µL of the amplified DNA, 150ng of each primer, 200µM of each dNTP, and 1U of Taq DNA polymerase. The first cycle will consist of 5 minutes at 95°C, then 35 cycles (each consisting of steps at 95°C for 1 minute, 64°C for 1 minute, and 72°C for 1 minute), and 9.5 minutes at 72°C to complete extension. The amplified DNA (10µL) will be digested with 10U NlaIII at 37°C for 12 hours and separated by electrophoresis on 8% polyacrylamide gel. After staining with ethidium bromide, DNA bands will be visualized under UV light. For homozygous FCγRIIIa-158F/F patients, only one undigested band (94 bp) should be visible. Three bands (94 bp, 61 bp, and 33 bp) should be seen in heterozygous individuals, whereas for homozygous FCγRIIIa-158V/V patients only 2 digested bands (61 bp and 33 bp) should be obtained. Similarly, genotyping of FCγRIIa-131-H/R will consist of PCR followed by allele-specific restriction enzyme digestion, also described by Cartron, et al.[22]

Statistical Analysis

The primary endpoint of the clinical trial within this study is complete response. The secondary endpoint is overall response (complete or partial). Statistical analysis of the clinical

and molecular responses of the patients in the different genotypic groups will be compared by a two-tailed Fisher exact test, as well as an exact trend test (i.e., permutation version of chi-square test for trend). The latter trend test examines the association of number of alleles (0, 1 or 2) with the endpoint. A logistic regression analysis including age >60, stage III or IV, bulky lymphadenopathy greater the 6cm, bone marrow involvement, number of extranodal sites >2, B2M, will be used to identify independent prognostic variables influencing clinical and molecular response.

A total of 39 patients will be studied. We shall have sufficient power (i.e., 80%) to detect associations of complete response with presence of an allele (e.g., 1 or 2 copies vs 0) whose rate ratio is 3.5. For instance, if in truth 75% of patients with 1 or 2 copies have complete response versus 20% with 0 copies we shall have 80% power to obtain a significant finding (i.e., p<=0.05). This calculation assumes a complete response rate of 35% and genotype frequency of approximately 2 alleles: 15%, 1 allele: 45% and 0 alleles: 45%.

Expected Results

We anticipate finding an independent correlation between the FcgammaRIIIa-158/FcgammaRIIa-131 polymorphisms and response. These results will define the effects of rituximab immunotherapy on follicular lymphoma, and learn the effects of rituximab within a combination regimen that includes bendamustine, a novel highly active drug. If this pilot study shows a correlation of these FcγR polymorphisms with response, it will substantiate findings by others that rituximab primarily acts through ADCC. Our results will be the first reported for rituximab in combination with the novel agent bendamustine. If this pilot study does not show a correlation with response rate, it may suggest that an alternative endpoint be used to correlate with these polymorphisms such as progression-free survival.

Alternative Studies

Alternatively, if no correlation is found between these polymorphisms and response, it would suggest that rituximab acts by other mechanisms in combination with chemotherapy and these mechanisms would need to be explored further. For instance, FcγRI may play a role in rituximab immunotherapy. FcγRI binding and its contribution to ADCC may be enhanced by cytokines such as IL-3, by qualitative effects on binding (termed “inside-out” regulation).[31] Apart from ADCC, a CDC mechanism directed through C1qA may also play a role in long-term response and PFS in rituximab-treated follicular lymphoma patients.[32] Pharmacogenetics relating to bendamustine therapy such as CYP1A2[33] or DNA repair enzyme polymorphisms[34, 35] may play a role in the responses to bendamustine. When used in combination with chemotherapy, rituximab may prime malignant cells to the effects of cytotoxic chemotherapy, as well as overcome chemotherapy resistance.[36] Rituximab may modulate the chemosensitivity of follicular lymphoma tumor cells by downregulation of Bcl-2 and Bcl-XL[36] and this phenomenon would need to be explored further. Polymorphisms or mutations of the CD20 protein may correlate with rituximab sensitivity or resistance,[37] and can be further studied. Study of these alternative factors will enhance our understanding of targeted immunotherapy for follicular lymphoma.

Feasibility

An accrual of 39 patients is planned for D1015 over 2-3 years. This is a multicenter trial with an expected accrual rate of 1-2 patients per month. In one year, we expect 12-15 patients assessable for clinical and molecular response. The preliminary correlations between polymorphisms and response obtained from this study will be used for future grants.

Relevance of results to cancer and translational merit

The use of FcγRIII and FcγRII polymorphism testing to predict outcomes of follicular lymphoma patients treated with rituximab in combination with chemotherapy has the capacity to contribute to the individualized care of the patient. Predictive genomic testing can play an integral role in choosing treatments that are highly effective and omitting agents that are not likely to have any effect. *Successful demonstration of an association between FcγRIII and FcγRII polymorphisms and response to this novel combination of rituximab and bendamustine will enable predictive genomic testing for follicular lymphoma patients treated with this regimen.*

Future Directions

There are now a new generation of anti-CD20 mAbs including ofatumumab and GA101. Ofatumumab is a fully humanized anti-CD20 mAb which is approved for use in refractory chronic lymphocytic leukemia, and is an exploratory treatment for follicular lymphoma. Ofatumumab binds to a different epitope of CD20 compared to rituximab, and its mechanism of action is unknown, but likely functions through ADCC and/or CDC. A multicenter collaborative clinical trials working group has been established that includes Dartmouth, Brown, and Yale. One of the ongoing clinical trials from this working group is studying the clinical response of ofatumumab combined with bortezomib in patients with low-grade B-cell NHL. The differences in immune mechanisms of rituximab and ofatumumab activity have not been well studied. The results and methods established from the current pilot proposal can be applied to the next generation CD20 mAbs to understand these differences.

This pilot data would serve as the framework for extramural funding for these additional studies.

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APPENDIX E

Karnofsky and ECOG Performance Status Conversion Table

ECOG	KPS	Performance Status
0	90-100	Fully active, able to carry on all pre-disease performance without restriction.
1	70-80	Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature.
2	50-60	Ambulatory and capable of all self care but unable to carry out any work activities. Up and about more than 50% of waking hours.
3	30-40	Capable of only limited self care, confined to bed or chair more than 50% of waking hours
4	10-20	Completely disabled. Cannot carry on any self care. Totally confined to a bed or chair.
5	0	Dead

APPENDIX F

Cockcroft-Gault Equation for Creatinine Clearance:
Creatinine clearance is calculated from plasma creatinine (Cr), age, weight, and gender correction factor (GF; 0.85 in women, 1.0 in men).

Cockcroft-Gault GFR = (140-age) * (Wt in kg) * (0.85 if female) / (72 * Cr)

APPENDIX G

In order to correctly assess lymphoma response, please follow the guidelines:

1. Determine lymphoma measurable disease
    a. Select a maximum of 6 lesions with the following features:
        i. Measurable in at least 2 perpendicular dimensions
        ii. Representative of all involved organs/areas
        iii. Suitable for accurate repeat measurements
        iv. Include mediastinal and retroperitoneal areas of disease
2. Determine lymphoma non-measurable disease
    a. Assessable Disease
        i. Masses that cannot be measured bi-dimensionally
        ii. Organ involvement (liver and spleen)
        iii. Recorded as "present" at baseline, then "no change", "increased", "decreased", "absent" at follow up
    b. Bone marrow status
        i. Positive or negative for lymphoma cells
3. Report bi-dimensional nodal measurements
    a. Select sites and measure two longest diameters
    b. Multiply each node's two longest diameters together
    c. Sum the Products of the greatest transverse Diameters (SPD or tumor size)
    d. Double check calculations
    e. Use correct units (cm)
4. Calculating change:
    a. For response: % Decrease=(baseline-current disease assessment)/baseline
    b. For progression: % Increase=(current disease assessment-smallest sum)/smallest sum

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